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NUCLEIC ACIDS AND THE GENESIS OF CANCER*

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A YEAR ago, a group of us at the Sloan-Kettering Institute and at the National Institutes of Health[1] isolated a nucleic acid from polyoma virus-infected mouse cells which proved to be infectious to cells in culture and, with lower frequency, in whole animals. The polyoma virus induces a large variety of tumors in rodents.[2] As Vogt and Dulbecco have shown, the intact virus either is cytopathogenic in mouse or hamster cells in culture and leads to the production of more virus, or it can cause a transformation of these into tumor cells which proliferate upon injection into animals.[3] The isolation from virus-infected cells of a deoxyribonuclease-sensitive, ribonuclease-resistant, nucleic acid which produces the same type of cytopathology or tumor picture as does the intact virus indicates that the nucleic acid of this virus is of the deoxyribo or DNA rather than of the ribo or RNA type.[1] Smith and Dulbecco have recently presented independent evidence with

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purified polyoma virus which confirms the DNA nature of the nucleic acid,[4] and Weil has confirmed the infectious properties of this DNA.[5]

The findings reveal that the infectious, carcinogenic principle of the virus is DNA, and that it is this which entered the cell to cause cytopathogenic changes or to induce tumor formation. Although this is the first example of an infectious and indeed of a carcinogenic DNA, the Shope papilloma virus has also been found to contain DNA.[6] There has been a recent, brief report of the isolation of an infectious nucleic acid, probably DNA, from Shope papilloma virus.[7]

A ribonuclease-resistant nucleic acid, isolated from mouse cell cultures infected with BB-T_2 virus (a virus very similar to, if not identical with, the polyoma virus), has also been found to exert a cytopathogenic effect in mouse cell cultures which then induce sarcomata of bone and kidney on injection into newborn rats.[7a] Malignant tumors have been induced in mice following their injection with as yet unidentified nucleic acids which had been isolated from mouse and human tumors.[8, 9]

It is not known whether all tumor-inducing viruses contain DNA. There have been many attempts to isolate the infectious principle from the Rous sarcoma virus, to test its sensitivity to a specific nuclease, but this has not yet succeeded.[10] The question of the identity of the nucleic acid type in tumor viruses is of considerable interest since it is now generally believed that the genetic determinants ("genes") of cells are composed of DNA.[11, 12]

We do not understand yet why it is that the intrusion of the foreign DNA into the mouse or hamster cell produces the effects we observe. But enough is now known about the interaction of other nucleic acids and cells, and of the presence of DNA in cells in many systems to allow us to infer that the derangement we call cancer is intimately bound up with nucleic acids.[13-15]

A culture of normal mouse embryo cells yields DNA on extraction which, as far as we can tell today, is physically and chemically indistinguishable from that isolated from polyoma virus-infected cells. Yet one preparation is carcinogenic and the other is not.[1] In order to consider how they can differ and whether any differences are pertinent to the problem of carcinogenicity, it is necessary to know just what kind of molecules the nucleic acids are and how they are distributed and function in nature. This is part of the general problem of the chemical basis of the biological activity of nucleic acids.

All cells which are capable of division and which have been examined for this purpose have thus far been found to contain both DNA and RNA.[16, 17] Unlike the RNA, the DNA content of the diploid cells during interphase is remarkably constant for the various tissues of the mammal, but there are severe alterations in polyploid and cancer cells; in any case, the DNA content *per chromosome set* appears to be quite constant.[13, 18] The number of DNA molecules per human diploid nucleus can be estimated to be about 800,000 from the DNA content[16] and from the assumption that the average molecular weight is 6,000,000.[19, 20] On this basis, and the fact that there are 46 chromosomes per normal human cell,[21] there would be very roughly about 20,000 molecules of DNA per human chromosome. In contrast, bacterial cells contain only a few hundred molecules of DNA, but many more of RNA.

The viruses are distinct from cells in many ways, but, for the purpose of this discussion, it is pertinent to point out that the most highly purified viruses contain either DNA, or RNA, but not both. The plant viruses thus far studied contain only RNA, and usually only a single molecule of RNA of molecular weight about 1.5 to 2 million, which amounts to about 5 to 44 per cent of the mass depending upon the virus.[22] The bacterial viruses, or phages, examined thus far contain only DNA, to the extent of 25 to 50 per cent of the weight of the virus; some of these contain but a single molecule of DNA whilst others may contain as many as 10 or perhaps 20 molecules.[23] The animal viruses are either of the DNA or RNA type. The Shope papilloma virus, for example, is spherical, with a particle weight of 47×10^6 containing about 8 per cent DNA by weight. If all the DNA within one such virus particle were present as a single molecule, the DNA would have a molecular weight of about 4×10^6; the molecular weight determined for the DNA after isolation is somewhat larger.[6]

The available evidence strongly suggests that the genetic identity and biological activity of viruses are determined only by their nucleic acid components (see 22, 23 for extensive references). In the infection of a susceptible bacterial cell by a T-even phage, the virus itself does not penetrate and enter the cell; rather, the bulk of the viral particle (the protein "head") remains outside the bacterium while all the DNA is "injected" into the cell.[23, 24] The transfer of the DNA from the phage to the bacterium constitutes a transfer of genetic material from the virus to the cell, and the changes which result thereby illustrate the

types of biological and biochemical activities which are associated with DNA. There are obvious changes such as the production by the invaded cell of a hundred or more virus particles rather indistinguishable from the original, and there is concomitant lysis and death of the cell. Since the DNA and the several types of proteins present in the phage do not occur normally in the bacterial cell, it is clear that the intruding DNA has endowed the infected cell with the ability to carry out a host of new biosynthetic reactions leading to many new macromolecules and finished viruses. These new biosynthetic reactions in turn depend upon the formation of many new enzymes and certain inhibitory substances which block synthesis of some of the normal bacterial nucleic acids; a new type of RNA is apparently formed. In addition, there is a considerable increase in the amount and activity of many pre-existing enzymes.[23, 25-28]

It can thus be said that the intrusion of the DNA from a single vegetative virus particle into a bacterial cell which already contains about one hundred or more times as many host DNA molecules is enough to divert the normal physiology towards the production of a great many new substances culminating in the appearance of genetically specific molecules and particles.

In certain situations, the insertion of the viral genome into a susceptible cell does not lead to the direct formation of more virulent phage. Rather, the viral DNA appears to remain in a dormant condition attached to the bacterial genome. This combination of genetic material can persist for many cell generations and is reproduced when the cell reproduces. This situation, which is referred to as lysogeny and which involves a temperate or latent virus state called prophage, can revert to the virulent condition by application of ionizing radiations, or carcinogenic or mutagenic agents.[23, 29] Thus, a nucleic acid can persist in the bacterial chromosome and exert a genetic effect for many generations of cells without the manifestation of a disease process until the proper provoking event triggers the production of new virus particles. The prophage is endowed with genetic continuity and is also capable of undergoing mutation like other chromosomal components. What, then, is the origin of the virus? Is it truly an extracellular agent or is it an ingenious mechanism for the transfer of genetic determinants from one cell to another?

The transfer of genetic determinants may be effected in animal and microbial systems by such devices as mating and viral infection. How-

ever, as we have seen above, exposure of cells to subviral agents such as the DNA of the polyoma virus is still another means of accomplishing this. This may be considered as an example of the transformation phenomenon, first described in 1944, in which hereditary determinants can be acquired by simple exposure of a receptive bacterial cell to the appropriate transforming DNA derived either from closely related bacterial strains or from different species.[11, 30] The bacterial transforming activity can be inhibited by DNA isolated from receptor cells and from mammalian sources as well.[30]

Although the phenomenon of bacterial transformation constitutes a most direct demonstration of the genetic activity of DNA, it can no longer be considered to be merely a laboratory-contrived exercise in genetic chemistry since the genetics of strains of *Neisseria* can be altered permanently by the DNA which occurs in the normal slimy exudate produced during the growth of the heterogenetic strains of these organisms.[31] Growing populations of *pneumococcus* also release into their cultures DNA capable of transforming other strains of pneumococci and this may therefore constitute an important natural mechanism of mutation or genetic recombination.[32] The possibility that nucleic acids may be extruded from mammalian cells is under investigation.

It is clear that the intruding nucleic acids cited in these several examples must have differed in some important regard (or regards) from those already present in the cell invaded by the DNA. It can be expected that elucidation of the pertinent differences may contribute to an understanding in chemical terms of how these nucleic acids function biologically. The truth is, of course, that we know practically nothing of the chemical basis of the biological activity of DNA. Much has been written concerning its chemical and physical properties and key citations to current knowledge may be found in several references at the end of this article.

The current picture of DNA is that of a long polymer of mononucleotide units held together by phosphodiester linkages. There are the "big four" mononucleotides, namely thymidylic, deoxyguanylic, deoxyadenylic and deoxycytidylic acid which occur in all DNA's studied except for the T-even bacteriophages which contain the 5-hydroxymethyl derivative of the last-mentioned nucleotide. Components of DNA and RNA are listed in Table I in which some recently discovered constituents are given together with some exceptions. As an average

TABLE I—KNOWN COMPONENTS OF NUCLEIC ACIDS

	Ribonucleic acids (RNA)	Deoxyribonucleic acids (DNA)
Sugars	D-ribose	D-2-deoxyribose
	D-2-methylribose*	
Phosphorus	Phosphate	Phosphate
Bases		
Major**	Adenine (6-aminopurine)	Adenine
	Guanine (2-amino-6-hydroxypurine)	Guanine
	Cytosine (2-hydroxy-4-aminopyrimidine)	Cytosine
	Uracil (2,4-dihydroxypyrimidine)	Thymine (5-methyluracil)
		5-Hydroxymethylcytosine
Minor†	5-Ribosyl uracil	
	6-Methylaminopurine	5-Methylcytosine
	6-Dimethylaminopurine	6-Methylaminopurine
	2-Methyl adenine	
	1-Methyl guanine	
	2-Methylamino-6-hydroxypurine	
	2-Dimethylamino-6-hydroxypurine	
	Thymine	
	5-Methylcytosine	
Artificial‡	2-Thiouracil	5-Chlorouracil
	8-Azaguanine	5-Bromouracil
	6-Azauracil	5-Iodouracil
	5-Fluorouracil	2-Amino-6-mercaptopurine (?)

*A recently discovered minor sugar component of RNA from several animal and plant sources; identity is probably correct.

**The major bases occur in all RNA's and DNA's respectively, except for cytosine which is not present in the DNA's of T-even bacteriophages and which is replaced, *only in those viruses,* by 5-hydroxymethylcytosine or a glucosidated derivative thereof.

†The minor constituents do not appear to occur in RNA-containing viruses. Several of these bases are more abundant in the "soluble" or transfer RNA's, which are concerned with activation of amino acids for protein synthesis; in any case, these minor bases together usually do not account for more than 1 or 2% of the total bases.

‡These "unnatural" constituents, which in some instances may replace most of the normal bases to which they are structurally related, have resulted from the deliberate addition of the analog to a biological system. Aspects of this type of replacement in RNA and DNA are given in Matthews, R. E. F., *Pharmacol. Rev. 10*:359 (1958).

Excluded from this list are cations and amino acid, peptide or protein constituents which may be integral components of some nucleic acid preparations.

nucleotide (a monophosphate of a sugar linked to a base) may have a molecular weight of roughly 320, a DNA which often contains 20,000 nucleotide units will show a molecular weight of some 6 million.[19] Wide agreement on the exact molecular weight of such macromolecules is lacking, but in any case DNA is certainly among the largest of molecules in nature. The current belief is that genetic information inherent in DNA is due to a specific sequence or arrangement of the mononucleotide units along the polymer chain, and much effort is being

expended in unravelling this genetic code. It is believed that genetic differences reside in differences in the sequence of mononucleotides. The problem of nucleotide sequence is currently among the most formidable in biological chemistry for several reasons.

For example, a polynucleotide consisting of a total of *only* 1,000 units, i.e., 250 of each of the four nucleotides mentioned above, could theoretically exist in about 10^{590} possible isomeric arrangements.[32] If but a single molecule of each of these possible polymers existed, the mass would total 10^{574} grams! The mass of the earth is estimated to be only 10^{27} grams,[33] hence certain isomers would probably not exist. But, real molecules of DNA are much larger than the hypothetical example given, hence the unravelling of the sequence imposes an extremely serious burden on the investigator who is interested in such explorations. It requires the preparation of DNA in such a condition of purity that *all* the molecules in the specimen be identical in so far as sequence is concerned. It is not certain whether the cell can replicate DNA molecules with this degree of precision, or even whether the maintenance of biological continuity requires precise replication of the total sequence.[35] Examples of inexact duplication of DNA have been discussed elsewhere,[36] and types of artificial analogs that can replace normal components of DNA *in vivo* are listed in Table I.

Although the replacement of thymine by its close structural analog 5-bromouracil in DNA has been found to be mutagenic in other bacteria and phages, molecules of DNA from *B. subtilis* retain their genetic transforming activity even after such extensive replacement.[37] In this instance, large recognizable differences between the analog and normal DNA's could be anticipated, yet these do not appear to affect significantly the biological activity which was measured.

The structure of a hypothetical fragment of DNA, consisting of eight mononucleotide units and hence having a molecular weight of about 2,500, is depicted in Figure 1. This formula is an adaptation of a double-stranded variety of DNA proposed by Watson and Crick[38] and consists, as shown, of two tetranucleotides coupled by arbitrarily selected pairs of hydrogen bonds linking (reading down from the top) adenine to thymine, thymine to adenine, guanine to cytosine, and, again, guanine to cytosine. A naturally-occurring DNA of which this (Figure 1) might well be a part would perhaps be 2,000 times as large, or larger. The available evidence indicates that, the -phosphate-deoxyribosyl-

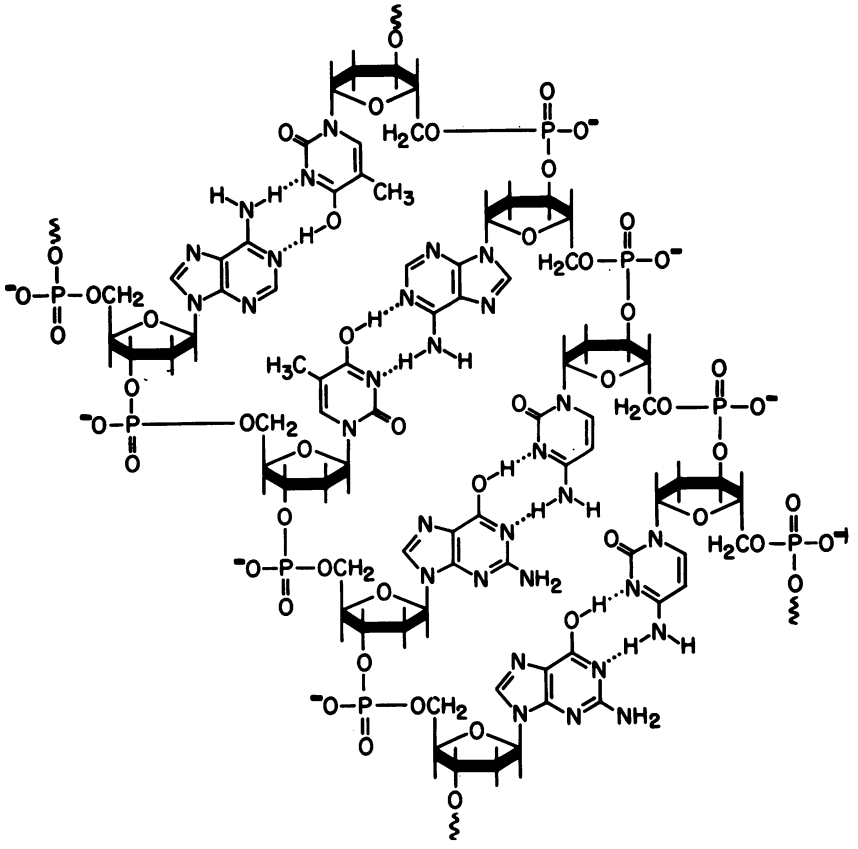

Fig. 1. Hypothetical fragment of double-stranded DNA, molecular weight about 2,500; actual molecules of DNA may possess molecular weights of several millions. In this approximate formulation, the guanine is shown linked to the cytosine by two hydrogen bonds; there may actually be three.

phosphate-deoxyribosyl- repeat backbone chain is identical for DNA from all sources but that the base (i.e., nucleotide) sequence differs. DNA's from many different sources have base compositions in which the ratios adenine:thymine as well as guanine:cytosine are unity or very nearly unity. Unity ratios are expected from complementary structures such as the one shown in the double-strand model in Figure 1, and this, in fact, was an important argument in the formulation of the Watson-Crick hypothesis. Not all DNA's show complementary base ratios and some, such as the DNA from the bacterial viruses ΦX-174 and S13, are single-stranded.[23]

Earlier studies[39] suggested that individual mammalian tissues con-

tained at least two types of DNA and that these differed chemically and metabolically. The scheme used to separate the two DNA fractions was a crude one and improved means of fractionation were sought. Figure 1 indicates that DNA is negatively charged, there being at least as many anionic charges as there are mononucleotide units. If the hundreds of DNA molecules in a bacterial cell or the hundreds of thousands in a mammalian cell were not all alike, they should constitute a mixture of different polyanions. Accordingly, an anion exchange chromatographic method was developed[36] which affected a partial separation of the DNA into many fractions.[40] Those from the DNA of calf thymus, for example, were found to differ in molecular size, and it was demonstrated that the fractions pre-existed in the original DNA which had been subjected to the chromatographic separation.[41] The chromatographic fractions differed in chemical composition, several showing noncomplementary base ratios and considerable single-stranded character. Fractions from multiply-marked pneumococcal transforming DNA differed in transforming activity, whilst some from nontransforming DNA and from calf thymus differed in the extent to which they inhibited the activity of transforming DNA.[42] Thus, the total genetic expression of these carriers of genetic information can be influenced by the composition of the mixture, and study of total, unfractionated DNA from a cellular source might give potentially misleading results. Fractions of DNA also differed considerably in the extent of their response to a variety of mutagenic and carcinogenic agents such as alkylating agents, ionizing radiation, and heat.

Typical examples of the complexity of the mixtures of fractions in human tissue are given in Figure 2 which represents the anion exchange chromatographic profiles of the DNA from normal and leukemic leukocytes and normal spleen.[20] The resolving power and reproducibility of the method, discussed in detail elsewhere,[20, 35, 36, 40] permitted the conclusion that the chromatographic profiles of the DNA from normal and leukemic leukocytes were significantly different.[20] The question arises as to whether these chromatographic differences are pertinent to the biological differences between the normal and cancer cells. If it were possible at present to affect a DNA-mediated transformation of one type of leukocyte to the other, it would be feasible to determine which fraction or fractions were responsible and a concerted effort made to learn the chemical basis of this activity.

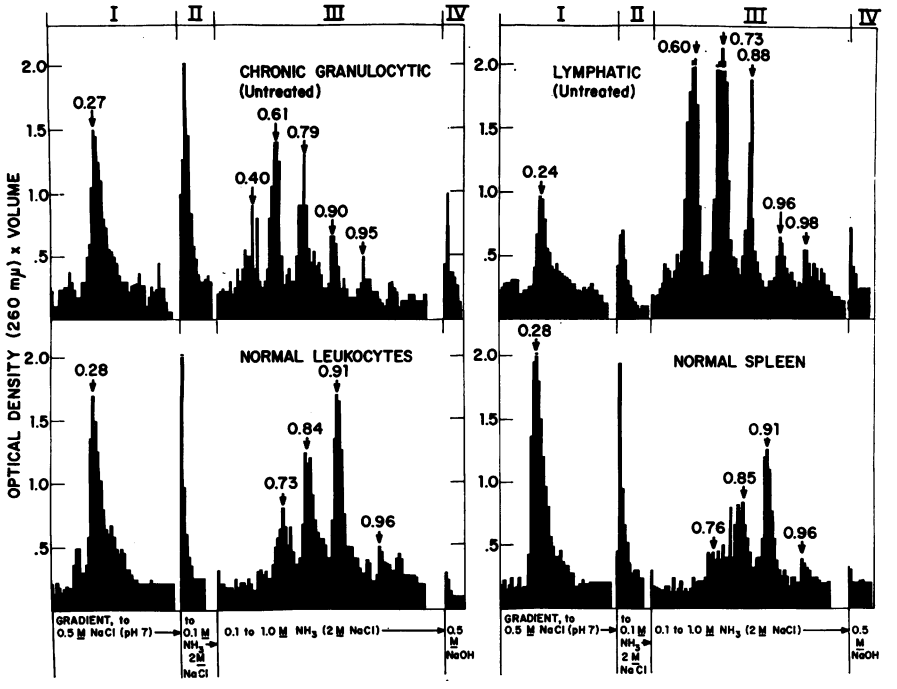

Fig. 2. Chromatographic profiles of DNA from normal human tissues and from the leukocytes of patients with chronic granulocytic and lymphatic leukemia (see reference 20). The numbers above the peak in Region I refer to the molarity of NaCl, and those above Region III refer to the molarity of ammonia, used to effect desorption of fractions of DNA from chromatographic columns containing the DNA adsorbed on an anion exchanger known as ECTEOLA. The areas shown in black are directly related to concentration of DNA.

It is just possible that the many failures thus far to transform a normal to a tumor cell with DNA from a cancer cell might have been due to the type of DNA-induced inhibition of bacterial transformation mentioned above, but that tumor induction could be mediated by the appropriately selected fraction free of inhibitory DNA. Perhaps the infection of mouse cells with the carcinogenic DNA from polyoma virus succeeded because the virus is devoid of inhibitory DNA as the virus may contain only a single molecule. Once tumor-cell formation results from cellular penetration of the carcinogenic DNA it may be reproduced in succeeding generations of tumor cells and might show up as an innocuous-looking small peak in a chromatographic profile as in Figure 2.

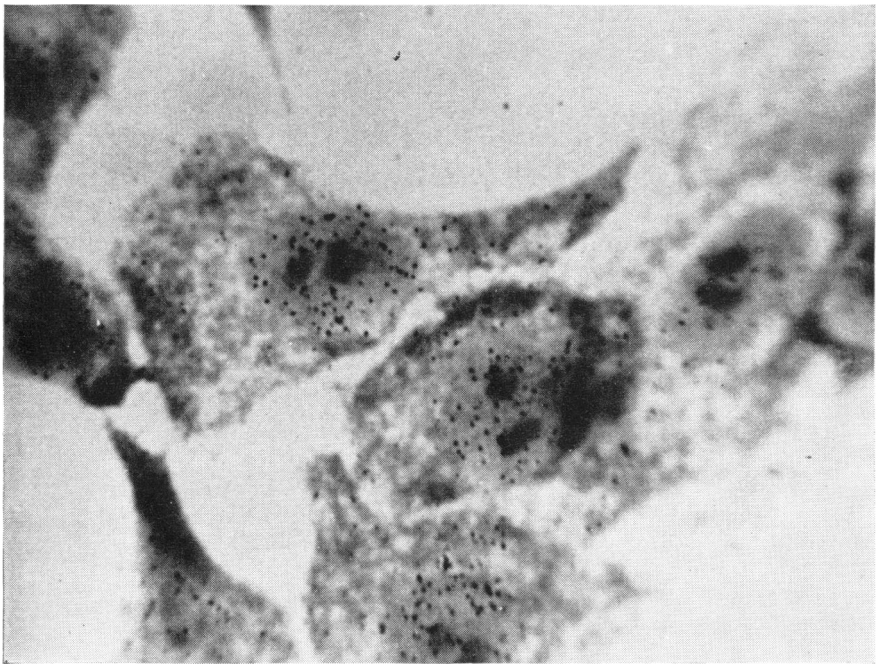

Fig. 3. Autoradiograph of human (HeLa) cells grown for 24 hours in the presence of tritium-containing DNA from human leukemic leukocytes (see reference 44). McNeals stain. X 1000

These speculations and questions are being examined experimentally. One of the approaches involves development of a model system in which to study the penetration of mammalian cells by DNA. To facilitate observations of the fate of and the effects produced by the intruding nucleic acid, DNA was labeled[43] with the radioactive isotope of hydrogen, tritium, since this is the most suitable for cytochemical and autoradiographic examination.

Solutions of tritiated DNA from human leukemic leukocytes were added to growing cultures of human (HeLa) cells. After various periods of time, the cells were washed and fixed for autoradiography before and after specific chemical or enzymatic treatment.[44] An example of a typical autoradiograph is shown in Figure 3 in which the tritium labels are detected by the presence of the small black silver grains within a distance of one micron (10^4 Å) of their actual location in the cell. These experiments reveal that the radioactive label in the

tritiated DNA had penetrated the cell and had localized largely in the nucleus. The label, prior to autoradiography, could not be removed from the cells either by washing with dilute acid or by treatment with RNase. However, it was removed by DNase, hence the label within the cells revealed by the silver grains was associated with macromolecules with properties expected of DNA. A similar picture is seen with fractions of human DNA and with a multiply-marked transforming DNA from pneumococcus,[44] and the biological effects of this type of infection are being investigated. The question whether the molecules which had penetrated and deposited in the nuclear regions were identical with those to which the cells were exposed is being studied in reisolation and fractionation experiments. Nontritiated DNA was found to inhibit the uptake of tritium label.

This model system lends itself to a study of the factors which can affect the cellular adsorption, transfer and deposition of DNA from normal and cancer tissues. It is hoped these studies will contribute to a further understanding of the role the nucleic acids appear to play in the genesis of cancer.

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